Biochemical and Biophysical Research Communications 409 (2011) 52–57

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Rad GTPase induces cardiomyocyte apoptosis through the activation of p38 mitogen-activated protein kinase

Zhongcui Sun [a,b], Ji Zhang [b], Jifeng Zhang [c], Chunlei Chen [b], Quan Du [b], Lin Chang [c], Chunmei Cao [b], Ming Zheng [b], Minerva T. Garcia-Barrio [d], Yuqing E. Chen [c], Rui-Ping Xiao [b], Jieming Mao [a,*], Xiaojun Zhu [b,*]

[a] Department of Cardiology, Peking University Third Hospital, No. 49 North Garden Road, Beijing 100191, China
[b] Institute of Molecular Medicine, Peking University, No. 5 Yi He Yuan Road, Beijing 100871, China
[c] Cardiovascular Center, University of Michigan, 1150 W Medical Center Drive, Ann Arbor, MI 48109, USA
[d] Cardiovascular Research Institute, Morehouse School of Medicine, 720 Westview Drive SW, Atlanta, GA 30310, USA

ARTICLE INFO

Article history:
Received 18 April 2011
Available online 28 April 2011

Keywords:
Rad GTPase
Cardiomyocyte
Apoptosis
p38 MAPK
Bcl-x_L

ABSTRACT

Rad is a member of a subclass of small GTP-binding proteins, the RGK family. In the present study we investigated the role of Rad protein in regulating cardiomyocyte viability. DNA fragmentation and TUNEL assays demonstrated that Rad promoted rat neonatal cardiomyocyte apoptosis. Rad silencing fully blocked serum deprivation induced apoptosis, indicating Rad is necessary for trigger cardiomyocyte apoptosis. Rad overexpression caused a dramatic decrease of the anti-apoptotic molecule Bcl-x_L, whereas Bcl-x_L overexpression protected cardiomyocytes against Rad-induced apoptosis. Rad-triggered apoptosis was mediated by the activation of p38 MAPK. The p38 blocker SB203580 effectively protected cardiomyocytes against Rad-evoked apoptosis.

1. Introduction

Rad (Ras-related associated with diabetes) is a 35-kD GTPase that belongs to the RGK (Rad, Gem, and Kir) family [1]. Members of this family share conserved GTP binding domains with Ras but lack the C-terminal CAAX motif involved in prenylation of Ras family members [2]. Rad was initially identified by subtraction cloning as overexpressed in skeletal muscle in a subset of patients with type II diabetes mellitus [2]. Significant efforts have been directed towards understanding the function of Rad in glucose metabolism. Insulin stimulated 2-deoxyglucose uptake decreased in Rad-overexpressing 3T3-L1 adipocytes or C2C12 myocytes [3]. Skeletal muscle specific Rad overexpression mice show normal glucose and insulin metabolism under regular diet, but show more severe insulin resistance under high fat diet [4]. Rad interacts with calmodulin and calmodulin-dependent protein kinase II, leading to inhibition to their downstream signals [5,6]. Local gene delivery of Rad attenuates neointimal formation after balloon injury by inhibiting vascular smooth muscle cells attachment and migration activated by the Rho kinase pathway [7]. Rad also influences remodeling of the cytoskeleton through inhibition of ROK [8]. Recently, increased attention has been paid to the functions of Rad protein in the heart. Our findings as well as the others' indicate that Rad inhibits myocardium L-type calcium channel activity and attenuates the β-adrenergic receptor (β-AR) activity [9,10]. Dominant negative suppression of Rad by a S105N mutant allele leads to QT prolongation and causes ventricular arrhythmia, suggesting a role for Rad signaling pathway in arrhythmia in diverse cardiac diseases [11]. Further more, our recent studies indicate that Rad expression decreases significantly in human failing hearts and Rad knockout mice are more susceptible to cardiac hypertrophy and cardiac fibrosis [12,13]. Those observations indicate that Rad expression is essential in maintaining normal cardiac function.

Emerging data suggest that small G proteins are implicated in the regulation of cardiac contraction, hypertrophy, and apoptosis [14]. Targeting small G proteins and their downstream signaling pathways may provide novel therapeutic approaches for the treatment of cardiovascular disorders such as heart failure, ischemic infarction, and hypertension [15,16]. Although much effort had been placed in understanding the functions of Rad in the heart, the effects of Rad in cardiomyocyte survival remain unknown. In the present study, we found that overexpression of Rad in cardiomyocytes induces cell apoptosis by activation of p38 MAPK and inhibition of Bcl-x_L expression.

2. Materials and methods

2.1. Adenoviruses

Recombinant adenoviruses for Rad overexpression or knockdown Ad-Rad and Ad-Rad-shRNA were generated as previously

* Corresponding authors. Fax: +86 010 8226 6699 (J. Mao), +86 010 6275 5291 (X. Zhu)
E-mail addresses: maojm518@yahoo.com.cn (J. Mao), zhuxiaojun@pku.edu.cn (X. Zhu).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.04.104

described [7,10]. Ad-Rad infection was performed at a multiplicity of infection (MOI) of 20 unless stated otherwise. An empty AdV was added in the control groups to keep the amount of virus consistent. Cells were infected with 40 MOI Ad-Rad-shRNA to reach 70–80% knockdown of the Rad protein in cardiomyocytes. Ad-Ctrl-shRNA containing scrambled shRNA was used as control in Rad knock down experiments.

2.2. Cell culture

Neonatal rat ventricular myocytes were isolated and cultured as previously described [17]. The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). Experiments were approved by the Peking University Animal Care and Use Committee. The cells were incubated in serum-free medium for 24 h before initiation of each experiment unless stated otherwise.

2.3. DNA fragmentation assay

The cells were pelleted at 3000*g* for 6 min, washed once with PBS, and lysed at 50 °C in 500 μl lysis buffer containing 10 mmol/L EDTA (pH 8.0), 10 mmol/L Tris–HCl, and 150 mmol/L NaCl with 0.4% SDS and 100 μg/ml proteinase K for 3–5 h with gentle agitation. The solution was then extracted with phenol/chloroform/isoamyl alcohol, and precipitated at −80 °C overnight. After centrifugation at 12,000 rpm for 10 min, the precipitate was washed with 75% alcohol, air-dried, diluted with 50 μl TE and treated by RNase A at 37 °C for 30 min. Ten micrograms of DNA from each sample were separated by electrophoresis on 2% agarose gel. The DNA bands were then imaged by ethidium bromide staining and photographed.

DNA fragmentation was quantitated by the Cell Death Detection ELISA^{PLUS} kit (Roche Molecular Biochemicals) according to the manufacturer's instructions. The intensity of absorbance at 405 nm was measured with a microplate reader relative to that of the substrate solution (blank).

2.4. Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) staining

Cardiomyocyte apoptosis was evaluated with the DeadEnd Fluorometric TUNEL system according to the manufacturer's instructions (Promega). Briefly, cells growing on coverslides in 12 well dishes were infected with recombinant adenoviruses. Forty-eight hours after infection, cells were fixed in 4% paraformaldehyde for 25 min at 4 °C and then permeabilized with 0.2% Triton X-100 solution for 5 min. The cells were then incubated with terminal deoxynucleotidyl transferase and fluorescein-12-dUTP for 1 h at 37 °C and reactions were stopped by dipping slides in 2X SSC for 15 min. The cells were counterstained with DAPI for 15 min before observation with a fluorescence microscope. To calculate the percentage of apoptotic cells, 500–700 cells in 10 randomly chosen fields from each dish were counted for the percentage of apoptotic nuclei.

2.5. Western blot analysis

Western blot was performed as previously described [18]. Rabbit anti-Rad polyclonal antibody was a kind gift from Dr. Ronald Kahn. All the other antibodies were from Cell Signaling Technology except the anti-β-actin monoclonal antibody that was from Sigma.

2.6. Statistical analysis

Data are presented as mean ± SD. Differences between mean values were evaluated by Student's *t* test with values of $P < 0.05$ indicating a significant difference.

3. Results

3.1. Rad overexpression induces rat neonatal cardiomyocyte apoptosis

To investigate the functional role of Rad protein, we infected rat neonatal cardiomyocytes with Ad-Rad. Cardiomyocyte viability was measured by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay after Ad-Rad infection and compared with non-virus infected cardiomyocytes or Ad-Ctrl infected cells at the MOI and time indicated in the figure (Fig. 1A). Rad caused significant decrease in cell viability proportional to the virus amount and hence the level of Rad expression. Phase-contrast microscopy evidenced the presence of a high number Ad-Rad infected cardiomyocytes with the characteristic morphology of apoptotic cells. This included the loss of cellular contacts, appearance of cellular blebbing, and finally detachment of cells from the plate (data not shown). In contrast, most of the Ad-Ctrl infected cells remained attached to the plate, and the apoptotic phenotype was only observed in a few cells. Ad-Rad infection profoundly promoted cardiomyocyte apoptosis, as evidenced by severe DNA fragmentation revealed by DNA laddering assay (Fig. 1B). Non-virus infected and Ad-Ctrl infected cells showed only high molecular weight DNA whereas Ad-Rad infected cells showed extensive internucleosomal DNA fragmentation and reduction of high molecular weight DNA. DNA fragmentation was further quantified by cell death detection ELISA on the basis of antibody detection of histone and fragmented DNA. Forty-eight hours after Ad-Rad infection, a 2-fold increase in DNA fragmentation was observed in Ad-Rad infected cells compared with Ad-Ctrl infected cells (Fig. 1C). TUNEL staining for apoptotic cells was performed to further confirm the apoptotic effect of Rad. As shown in Fig. 1D, there is a dramatic increase in TUNEL positive cells in Ad-Rad infected cardiomyocytes compared with control adenoviruses infected cells. Thus, excessive Rad expression is sufficient to trigger cell apoptosis.

3.2. Rad is an essential mediator of serum deprivation induced apoptosis in cardiomyocytes

Our above experiments showed that Rad overexpression induced apoptosis. To identify whether Rad contributes to cardiomyocyte apoptosis, we measured Rad expression level under serum deprivation. About 2-fold induction on Rad protein was observed in cardiomyocytes after 24 or 48 h of serum deprivation (Fig. 2A). To test the hypothesis that Rad is a necessary mediator for serum free induced apoptosis, we developed RNA interference (RNAi)-mediated loss-of-function experiments. Rad was successfully knocked down in cardiomyocytes by RNAi (Fig. 2B). Serum deprivation caused cardiomyocytes apoptosis and Rad knockdown completely rescued the apoptotic effect of serum deprivation as shown by DNA laddering (Fig. 2C), suggesting that Rad is not only sufficient but also essential for serum deprivation induced apoptosis.

3.3. Rad induced cardiomyocyte apoptosis through down-regulation of Bcl-x_L

Western blots were performed to detect changes in the Bcl-2 family members after Rad overexpression. There was a ~50% reduction on Bcl-x_L protein levels after 48 h infection with Ad-Rad (Fig. 3A), whereas the levels of Bax and Bcl-2 proteins remain

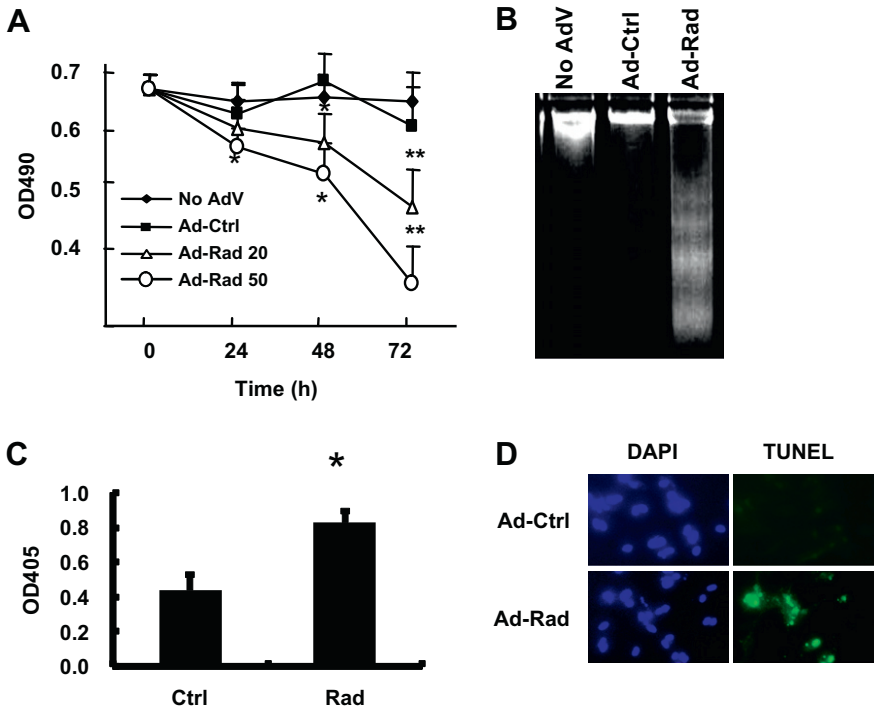

Fig. 1. Overexpression of Rad in rat neonatal cardiomyocytes results in reduced viability. (A) Time course of the cell viability as measured by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) after Ad-Rad infection. Ad-Ctrl was added to keep the amount of virus consistent at a MOI of 50 in the AdV infected groups. $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the Ad-Ctrl infected groups. (B) and (C) Enhanced DNA fragmentation after Rad overexpression. Rat neonatal cardiomyocytes were infected with Ad-Rad or control adenovirus. DNA fragmentation was analyzed 48 h after adenovirus infection by DNA laddering (B) and cell death ELISA (C). $^{*}P < 0.05$ vs. Ad-Ctrl infected group. (D) Rad markedly increased rat neonatal cardiomyocyte apoptosis assayed by TUNEL staining.

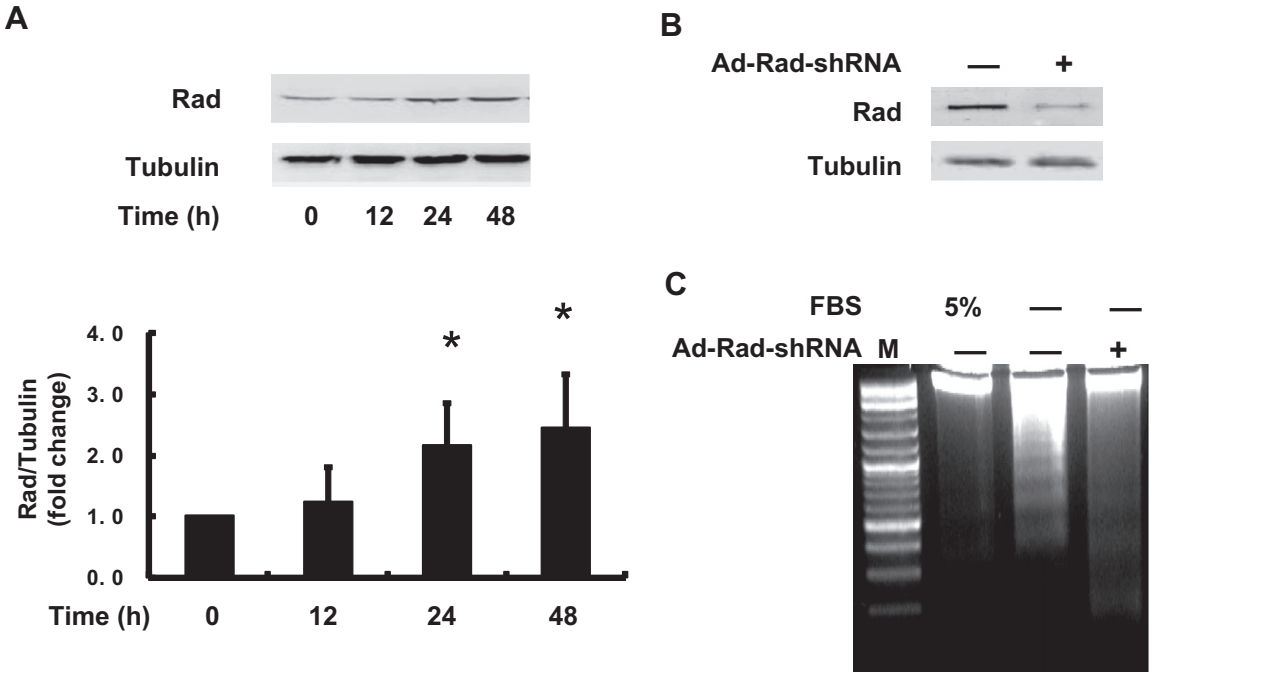

Fig. 2. Rad is an essential mediator of serum deprivation induced cardiomyocyte apoptosis. (A) Western blot was performed to detect Rad expression in cardiomyocytes after serum deprivation for the time indicated in the figure. Typical figures of at least 3 independent experiments and average data for the fold changes of Rad protein level normalized by Tubulin are shown. $^{*}P < 0.05$. (B) Ad-Rad-shRNA or Ad-Ctrl-shRNA was used to infect cardiomyocytes for 24 h. Rad knock-down after Ad-Rad-shRNA infection was confirmed by Western Blot. (C) Cardiomyocytes were infected with Ad-Rad-shRNA or Ad-Ctrl-shRNA before 72 h of serum deprivation. Cell apoptosis was detected by DNA laddering.

unchanged (data not shown). Then we further analyzed whether restoring Bcl-x_L was enough to protect cells from Rad induced apoptosis. Ad-Rad infection resulted in a dramatic increase in TUNEL-positive cells compared with the control adenovirus (23.4 ± 6.5% vs. 4.6 ± 2.8%), and Ad-Bcl-x_L infection at 20 MOI reduced the TUNEL-positive cells after Ad-Rad infection to 8.5 ± 4.4%.

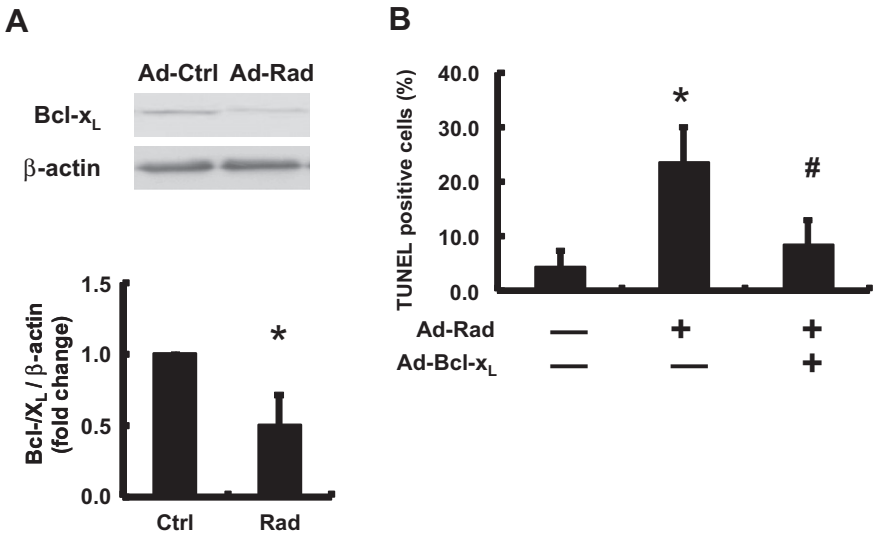

Fig. 3. Rad induces cardiomyocyte apoptosis via downregulation of Bcl-x_L. (A) Cardiomyocytes were infected with Ad-Rad or Ad-Ctrl and Western blots were performed to detect levels of Bcl-x_L. Typical Figures of 3 independent experiments and average data for the fold changes of Bcl-X_L protein level normalized by β-actin are shown. $^{*}P < 0.05$. (B) Cardiomyocytes were infected with recombinant adenoviruses as indicated in the figure and cell apoptosis was measured by TUNEL staining. $^{*}P < 0.05$ vs. Ad-Ctrl infected group, $^{\#}P < 0.05$ vs. Ad-Rad infected group.

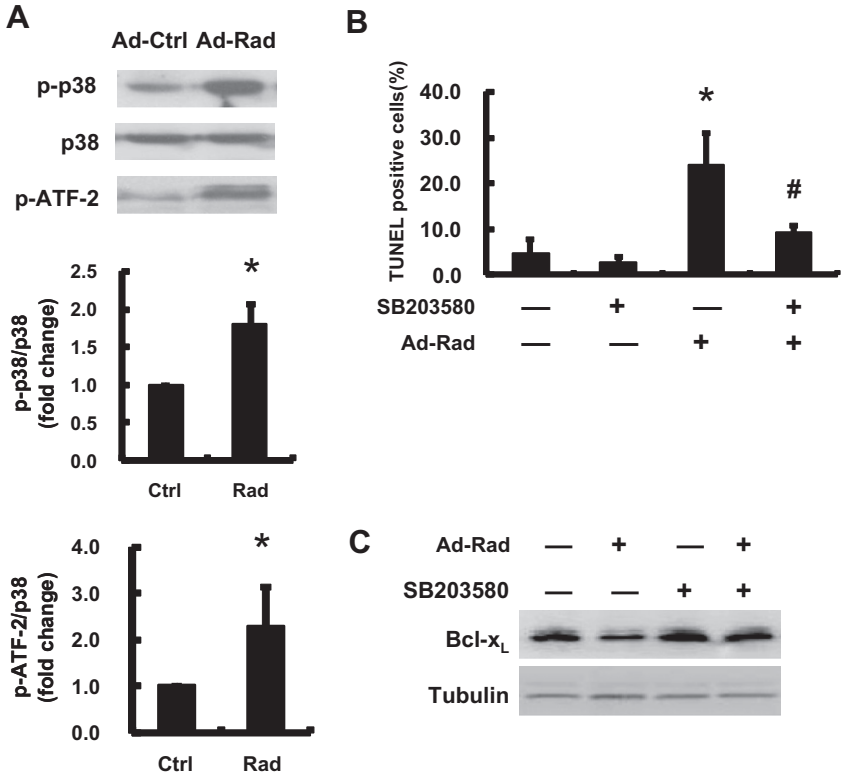

Fig. 4. Rad induces apoptosis through the activation of p38. Cardiomyocytes were infected with Ad-Rad or Ad-Ctrl and Western blots were performed 36 h after AdV infection to detect levels of p-p38, p38, and p-ATF2, respectively. Typical figures of 3 independent experiments and average data for the fold changes of protein level normalized by p38 are shown. $^{*}P < 0.05$. (B) SB203580 treatment protected cardiomyocytes from Rad induced apoptosis. Cells were treated with SB203580 at 10 μmol/L before Ad-Rad or Ad-Ctrl infection. TUNEL staining was performed to detect cell apoptosis. $^{*}P < 0.05$ vs. Ad-Ctrl infected group, $^{\#}P < 0.05$ vs. Ad-Rad infected group. (C) Bcl-x_L decreased after Rad expression and was restored by SB203580 treatment. Cells were treated with SB203580 at 10 μmol/L before Ad-Rad or Ad-Ctrl infection. Western blot was performed to detect Bcl-x_L.

3.4. p38 Mediates Rad induced cardiomyocyte apoptosis

Because of the significant role of MAPKs in cardiomyocyte apoptosis [19], we examined the effect of Rad overexpression on the activation of p38 MAPK. Western blot analyses demonstrated that Ad-Rad infection increased phosphorylation of both p38 and its downstream target ATF-2 (Fig. 4A).

We next investigated the contribution of p38 activation in Rad induced apoptosis. TUNEL assays indicated a marked reduction in Rad-induced apoptosis after pretreatment with a p38 inhibitor,

SB203580 (Fig. 4B). Western blot showed that downregulation of Bcl-x_L by Rad was almost completely prevented by SB203580 pretreatment (Fig. 4C). These data indicate that p38 pathway is the main component in Rad-mediated apoptosis.

4. Discussion

To date, more than 100 GTPases have been identified and they exert a wide spectrum of functions, including regulation of gene function, cell proliferation, migration, apoptosis and cytoskeleton rearrangement [16,20]. Rad had been cloned for over ten years but its functional role in myocardium had not been well defined. Previous reports documented that Rad is highly expressed in heart and skeletal muscle. We found that the expression of Rad is relatively much higher in cardiomyocytes than in cardiac fibroblasts [13], which led us to focus on the function of Rad in cardiomyocytes.

Apoptosis is important for organ development and adult tissue homeostasis and remodeling. The loss of terminal differentiated cardiomyocytes by apoptosis and followed replacement fibrosis play essential roles in the development of various heart diseases including heart failure and ischemic infarction [21]. Members of Ras small GTPase families have been demonstrated to induce apoptosis [22,23], but for the RGK family, a relationship with apoptosis has not been stated so far, especially in cardiomyocytes. This study appears to be the first to indicate that Rad induces apoptosis in neonatal rat ventricular cardiomyocytes through the activation of p38 MAPK, as well as downregulation of the anti-apoptotic protein Bcl-x_L.

There are currently two major paradigms for apoptotic cell death: the receptor-mediated pathway and the mitochondrial mediated pathway. The mitochondrial death pathway is initiated by cellular signals that affects the mitochondria, leading to the release of cytochrome *c* and caspase activation [24]. There is general agreement that a balance between pro- and anti-apoptotic Bcl-2 family proteins can directly regulate the mitochondria and determine cell fate [25]. Prosurvival Bcl-2 family proteins like Bcl-x_L are known to antagonize Bax-mediated mitochondrial membrane permeabilization, cytochrome *c* release and cell death. In the present study, we found that Rad overexpression resulted in the decrease of Bcl-x_L protein. In contrast, Bcl-2 and Bax were unaffected by Rad expression. Restoring Bcl-x_L protected the cardiomyocytes from Rad induced apoptosis thus further confirmed that Rad induced apoptosis through the mitochondrial pathway.

We demonstrated that Rad overexpression induced apoptosis via the phosphorylation of p38 MAPK. An intimate relationship between activation of p38 and induction of apoptosis in cardiomyocytes has been found in response to many apoptotic stimuli [26–28]. Although there are other small G protein members such as Rho family members that can activate p38 [29,30], it is the first time that a direct relationship was uncovered between RGK family members and the MAPKs. SB203580, a p38 inhibitor, rescued cells from apoptosis elicited by Rad overexpression. These data indicate that p38 activation is the main signal pathway responsible for Rad-induced apoptosis. However, the molecular mechanism underlying Rad-induced p38 phosphorylation needs to be further investigated.

We identified that Rad expression activated p38 and reduced Bcl-x_L and that a p38 blocker restored Bcl-x_L levels, indicating Bcl-x_L downregulation would be a consequence of Rad-induced p38 activation. It has been documented that Bcl-x_L decreases in heart after cardiac ischemia, while Bcl-x_L level is higher in mice with a cardiac specific expression of dominant-negative p38 compared with wild type mice [31]. Our observation adds to previous evidences suggesting a possible mechanism by which p38 mediates apoptosis via a crosstalk with the Bcl-2 family members [32].

In conclusion, we have demonstrated that increased levels of Rad induce cardiomyocyte apoptosis and that this effect is mediated by activation of p38 MAPK and the suppression of Bcl-x_L.

Acknowledgments

This work was supported by Peking University 985 Project; Natural Science Foundation of China (30400223 and 30671027); National Basic Research Program of China (2007CB512100 and 2010CB529503); American Heart Association National Career Development Grant [0835237N to J.Z.]; Midwest Affiliate Fellowship [0625705Z to L.C.].

References

[1] K. Kelly, The RGK family: a regulatory tail of small GTP-binding proteins, Trends Cell Biol. 15 (2005) 640.
[2] C. Reynet, C.R. Kahn, Rad: a member of the Ras family overexpressed in muscle of type II diabetic humans, Science 262 (1993) 1441–1444.
[3] J.S. Moyers, P.J. Bilan, C. Reynet, C.R. Kahn, Overexpression of Rad inhibits glucose uptake in cultured muscle and fat cells, J. Biol. Chem. 271 (1996) 23111–23116.
[4] J. Ilany, P.J. Bilan, S. Kapur, J.S. Caldwell, et al., Overexpression of Rad in muscle worsens diet-induced insulin resistance and glucose intolerance and lowers plasma triglyceride level, Proc. Natl. Acad. Sci. USA 103 (2006) 4481–4486.
[5] J.S. Moyers, P.J. Bilan, J. Zhu, C.R. Kahn, Rad and Rad-related GTPases interact with calmodulin and calmodulin-dependent protein kinase II, J. Biol. Chem. 272 (1997) 11832–11839.
[6] P. Beguin, R.N. Mahalakshmi, K. Nagashima, D.H. Cher, et al., Nuclear sequestration of beta-subunits by Rad and Rem is controlled by 14-3-3 and calmodulin and reveals a novel mechanism for Ca(2+) channel regulation. J. Mol. Biol. (2005).
[7] M. Fu, J. Zhang, Y.H. Tseng, T. Cui, et al., Rad GTPase attenuates vascular lesion formation by inhibition of vascular smooth muscle cell migration, Circulation 111 (2005) 1071–1077.
[8] Y. Ward, S.F. Yap, V. Ravichandran, F. Matsumura, et al., The GTP binding proteins Gem and Rad are negative regulators of the Rho–Rho kinase pathway, J. Cell Biol. 157 (2002) 291–302.
[9] B.S. Finlin, S.M. Crump, J. Satin, D.A. Andres, Regulation of voltage-gated calcium channel activity by the Rem and Rad GTPases, Proc. Natl. Acad. Sci. USA 100 (2003) 14469–14474.
[10] G. Wang, X. Zhu, W. Xie, P. Han, et al., Rad as a novel regulator of excitation-contraction coupling and {beta}-adrenergic signaling in heart, Circ. Res. 106 (2010) 317–327.
[11] H. Yada, M. Murata, K. Shimoda, S. Yuasa, et al., Dominant negative suppression of Rad leads to QT prolongation and causes ventricular arrhythmias via modulation of L-type Ca2+ channels in the heart, Circ. Res. 101 (2007) 69–77.
[12] L. Chang, J. Zhang, Y.H. Tseng, C.Q. Xie, et al., Rad GTPase deficiency leads to cardiac hypertrophy, Circulation 116 (2007) 2976–2983.
[13] J. Zhang, L. Chang, C. Chen, M. Zhang, et al., Rad GTPase inhibits cardiac fibrosis through connective tissue growth factor, Cardiovasc. Res. (2011), doi:10.1093/cvr/cvr068.
[14] J.H. Brown, D.P. Del Re, M.A. Sussman, The Rac and Rho hall of fame: a decade of hypertrophic signaling hits, Circ. Res. 98 (2006) 730–742.
[15] K. Budzyn, P.D. Marley, C.G. Sobey, Targeting Rho and Rho-kinase in the treatment of cardiovascular disease, Trends Pharmacol. Sci. 27 (2006) 97.
[16] J. Ren, C.X. Fang, Small guanine nucleotide-binding protein Rho and myocardial function, Acta Pharmacol. Sin. 26 (2005) 279–285.
[17] T. Shen, M. Zheng, C. Cao, C. Chen, et al., Mitofusin-2 is a major determinant of oxidative stress-mediated heart muscle cell apoptosis, J. Biol. Chem. 282 (2007) 23354–23361.
[18] X. Zhu, Y. Lin, J. Zhang, M. Fu, et al., Thiazolidinediones, a class of anti-diabetic drugs, inhibit Id2 expression through a PPARgamma-independent pathway in human aortic smooth muscle cells, Cell. Mol. Life Sci. 60 (2003) 212–218.
[19] C.P. Baines, J.D. Molkentin, STRESS signaling pathways that modulate cardiac myocyte apoptosis, J. Mol. Cell. Cardiol. 38 (2005) 47–62.
[20] L. Barki-Harrington, C. Perrino, H.A. Rockman, Network integration of the adrenergic system in cardiac hypertrophy, Cardiovasc. Res. 63 (2004) 391–402.
[21] A. Abbate, R. Bussani, M.S. Amin, G.W. Vetrovec, et al., Acute myocardial infarction and heart failure: role of apoptosis, Int. J. Biochem. Cell Biol. 38 (2006) 1834–1840.
[22] M. Ito, T. Adachi, D.R. Pimentel, Y. Ido, et al., Statins inhibit {beta}-adrenergic receptor-stimulated apoptosis in adult Rat ventricular myocytes via a Rac1-dependent mechanism, Circulation 110 (2004) 412–418.
[23] D.P. Del Re, S. Miyamoto, J.H. Brown, RhoA/Rho kinase up-regulate Bax to activate a mitochondrial death pathway and induce cardiomyocyte apoptosis, J. Biol. Chem. 282 (2007) 8069–8078.
[24] M.T. Crow, K. Mani, Y.-J. Nam, R.N. Kitsis, The mitochondrial death pathway and cardiac myocyte apoptosis, Circ. Res. 95 (2004) 957–970.

[25] Z.N. Oltvai, C.L. Milliman, S.J. Korsmeyer, Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death, Cell 74 (1993) 609–619.
[26] X.L. Ma, S. Kumar, F. Gao, C.S. Louden, et al., Inhibition of p38 mitogen-activated protein kinase decreases cardiomyocyte apoptosis and improves cardiac function after myocardial ischemia and reperfusion, Circulation 99 (1999) 1685–1691.
[27] A. Porras, S. Zuluaga, E. Black, A. Valladares, et al., P38 alpha mitogen-activated protein kinase sensitizes cells to apoptosis induced by different stimuli, Mol. Biol. Cell 15 (2004) 922–933.
[28] K. Mackay, D. Mochly-Rosen, An inhibitor of p38 mitogen-activated protein kinase protects neonatal cardiac myocytes from ischemia, J. Biol. Chem. 274 (1999) 6272–6279.
[29] S. Zhang, J. Han, M.A. Sells, et al., Rho family GTPases regulate p38 mitogen-activated protein kinase through the downstream mediator Pak1, J. Biol. Chem. 270 (1995) 23934–23936.
[30] S. Bagrodia, B. Dérijard, R.J. Davis, R.A. Cerione, Cdc42 and PAK-mediated signaling leads to Jun kinase and p38 mitogen-activated protein kinase activation, J. Biol. Chem. 270 (1995) 27995–27998.
[31] J. Ren, S. Zhang, A. Kovacs, Y. Wang, et al., Role of p38[alpha] MAPK in cardiac apoptosis and remodeling after myocardial infarction, J. Mol. Cell. Cardiol. 38 (2005) 617–623.
[32] J. Ren, S. Zhang, A. Kovacs, Y. Wang, et al., Role of p38[alpha] MAPK in cardiac apoptosis and remodeling after myocardial infarction, J. Mol. Cell. Cardiol. 38 (2005) 617.